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Abstract

Polarized transport of signaling molecules, such as the phytohormone auxin, is a core process for the establishment of gradients involved in the patterning of multicellular plant tissues. Cell-to-cell polarity of transport can result from the differential deposition of efflux carriers on the membranes at the interface between cells, as for the pin-formed1 (PIN1) auxin transporter. Quantifying such sub-cellular information in whole organ microscopy images constitutes a real challenge. In this work, we propose a method that computes polarities at the level of cell-to-cell interfaces starting from standard resolution confocal images and using a 3D geometric representation of cell walls. A robust estimation of spatial fluorescence distribution around cell walls allows quantifying polarity with a fair level of confidence, and opens the way for the automated analysis of polar transport at tissue scale.

Keywords: Quantitative Image Analysis, Confocal Microscopy, Triangle Mesh, Polar Transport.

1 Introduction

Cell polarity is a fundamental feature in developmental biology, where symmetry breaking is essential for the formation of patterns in multicellular organisms. In plants, along with anisotropic cell elongation or asymmetric cell division, polarity manifests notably by a preferred directionality in the intercellular flow of signaling molecules. In the preeminent case of auxin, polarized transport, mediated by the PIN efflux carriers, has been shown to play a determinant role in the establishment of early embryo apico-basal axis [1], aerial organ arrangement (phyllotaxis) [6], or leaf adaxial-abaxial axis [7]. It is the localization of such proteins at the plasma membrane on a preferential side of the cell that will increase the export of molecules to neighbor cells in a given direction, and locally orient the flow.

Live-imaging microscopy is used to monitor levels of transporters in a developing tissue, and to assess polarity of transport at cell-level. It is generally admitted that intracellular gradients mark the polarity of a transporter, and without reference for the cell wall position, the visual cue of a crescent shape on one side of a cell in 2D projections is often used to manually estimate cell polarities. However, in confocal images of transporters where the 0.1-0.2μm resolution exceeds cell wall thickness, it is impossible to visually assert which cell hosts the fluorescence, and ultimately to determine polarity. Recent works begin to rely on co-imaging to estimate the relative position of transporters and cell wall intensity peaks in 2D along user-specified lines [7].

Here, we propose a fully automated method to estimate polarities of transporters in confocal images using a cell wall marker reference and going beyond voxel resolution through the use of geometric representations detached from the image grid.

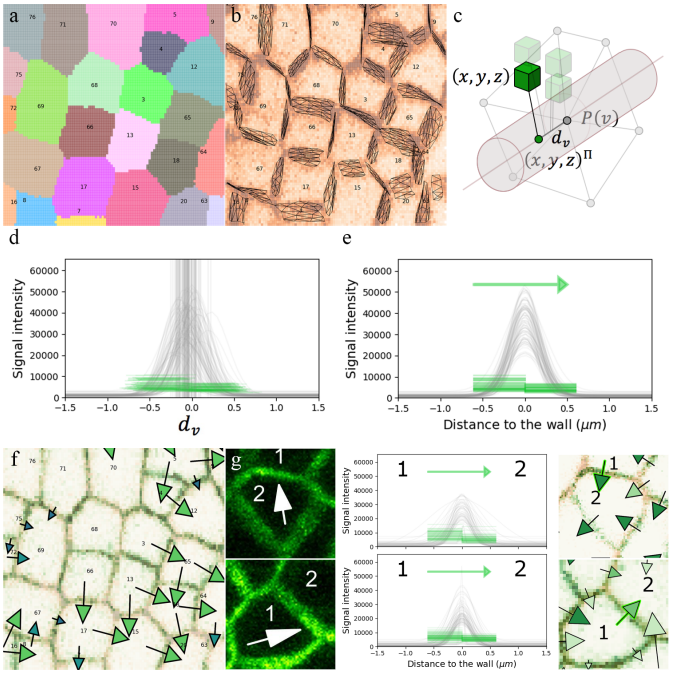

Figure 1: Wall-level quantification of transport polarity: 3D segmented image slice (a), projected wall meshes (b), locally orthogonal cylinder placed at each vertex of the wall and voxel projection (c), precise location of the cell wall intensity mode (d), left and right estimation of transporter levels and statistical polarity decision (e), tissue level polarity vectors (f), decorrelation between visual interpretation of intensity crescents and quantitative polarity estimation (g).

2 Wall polarity quantification

Our starting point consists of a 3D segmented image of multicellular tissue (Figure 1a), typically obtained by applying an automatic seeded watershed algorithm to a confocal stack of membrane or cell wall marker, where seeds can be detected as regional minima of signal [3]. Our polarity estimation algorithm has two steps : the extraction of cell walls as 3D triangular meshes, and the estimation for each wall of a polarity vector through the analysis of the local fluorescence distribution.

We use smooth triangular meshes to represent accurately the cell walls and limit the sensitivity to noisy segmentation and to image resolution. To obtain such meshes, we apply the Marching Cubes algorithm [5] to the binary image corresponding to each labelled cell region in the segmented image, and keep the common vertices for each pair of neighbor cells, using the triangulation of either of the two to define mesh elements. Those meshes undergo a phase of triangle decimation [4] and isotropic remeshing [2] to obtain a regular surface (Figure 1b). On the triangular mesh, we estimate the normal vectors at each vertex and the area of each triangle that allows us to estimate the total area of the interface between the two cells.

We consider that the transporter polarity vector of a given cell interface, i.e. whether the efflux carriers orient the flow of molecules towards one cell or the other, is given by the differential of concentration of transporters between the plasma membranes. We access this information through the difference of fluorescence intensity in the image on either side of the cell wall marked by the wall-marker intensity around each mesh.

We generate a set of 3D cylinders, placed at each vertex in the direction of the normal, in which we will sample the image signals. We position the image voxels lying inside this cylinder on an 1-dimensional axis by assigning them the signed abscissa d_v of their orthogonal projection on the main axis (Figure 1c). The position $d_v = 0$ (corresponding to the mesh vertex supporting the cylinder) might actually have shifted from the actual cell wall in the consecutive processing steps (segmentation artifacts, meshing simplifications, smoothing approximations). To account for this, we locate precisely the abscissa d_0 of the mode in the 1D wall-marker image intensity distribution by the least-squares fitting of a Gaussian-shaped function. Then, transporter levels are quantified on either side of this reference, up to a distance d_{max}, by computing the average voxel intensity within the two sub-cylinders (Figure 1d).

By performing this two-sided estimation on every cylinder defined by the wall triangular mesh, we end up with two parallel transporter signal distributions. We test statistically whether these distributions can be seen as significantly different by an ANOVA test, and decide that a polarity exists when the test gives a p-value < 0.05 (Figure 1e). In such case the polarity vector is given by the difference between the medians of the two distributions multiplied by the normal vector to the wall, otherwise it is null (Figure 1f).

3 Results & Discussion

We have applied our method to root images expressing fluorescent PIN2 and with stained cell walls using propidium iodide (PI). It is known that, in root epidermal cells, auxin transport is polarized shootward, and our method retrieves this expected polarity in all cells. We also studied the influence of spatial resolution and showed similar PIN1 polarities between shoot apical meristem (SAM) tissues imaged at 0.1μm and 0.2μm resolutions. Finally, a study on 16 SAM images reveals highly preserved patterns of PIN1 polarities, indicating that our method provides consistently reliable results at tissue scale.

Additionally, this 3D reconstruction of PIN1 polarities demonstrated that the crescent-shape often thought to indicate polarities in cells does not always correlate with polarities and can thus be sometimes misleading (Figure 1g). The method opens the way for a large scale study of accurate polarity dynamics at the scale of a whole tissue.

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